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(71) Applicant(s)
Wassen International Ltd
(Incorporated in the United Kingdom)
14 The Mole Business Park, LEATHERHEAD, Surrey, KT22 7BA, United Kingdom

(72) Inventor(s)
Raymond Sidney Matthews

(74) Agent and/or Address for Service
J A Kemp & Co.
14 South Square, Gray's Inn, LONDON, WC1R 5LX, United Kingdom

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(56) Documents Cited
FEBS Letters vol 421 (1998) pages 277-279 Cancer Research vol 57 (1997) pages 3649-3652 Science vol 275 (1997) pages 218-220

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(54) Abstract Title
Formulation comprising a brassica extract or sulforaphane and resveratrol

(57) The present invention discloses a composition suitable for pharmaceutical use which comprises at least one active ingredient from a brassica extract or an analogue of sulforaphane, and resveratrol or an analogue thereof. Suitably the composition comprises 1-100 mg of the brassica extract or sulforaphane analogue and 0.5-100 mg of resveratrol. Preferably the weight ratio of brassica extract to resveratrol is from 1:500 to 1:50. The composition may be used to treat cancer, especially testicular cancer.

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The claims were filed later than the filing date but within the period prescribed by Rule 25(1) of the Patents Rules 1995.

Formulation

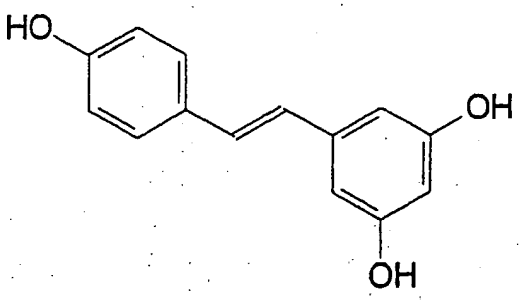
The present invention relates to compositions for retarding and/or preventing tumours.

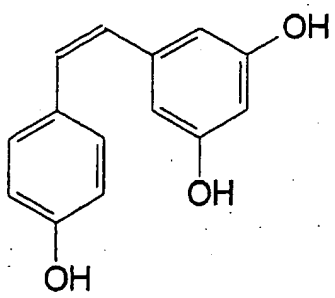
There have been numerous attempts at treating tumours. It is accepted that tumour development can be a multistage process, and that it can be inhibited by interfering with various discrete elements in the overall process. Inhibition of the earliest stages would generally be considered the most desirable protective effect.

Many compounds have been tested for their efficacy in preventing tumours. One such compound is resveratrol. *In vitro* experiments have been undertaken with resveratrol and there is evidence from *in vitro* cell culture and bacterial mutagenicity studies that resveratrol might retard tumour initiation when a mutagenic or carcinogenic challenge is given. The improved scavenging of free radicals, possibly by the induction of the phase II enzyme quinone reductase, has been proposed to account for the protection of the cells (Jang et al, *Science*, **275**, 218-20(1997)).

There is also evidence that resveratrol might inhibit cyclooxygenase, an enzyme considered to be involved in tumour promotion and various inflammatory conditions. Anti-proliferative effects of resveratrol have been noted *in vitro* and attributed to an inhibition of thymidine incorporation and the inhibition of esterase enzymes (Jang *et al.*, (1997)). In addition, in a leukaemia cell line, a marked inhibition of the enzyme ribonucleotide reductase has been reported (Fontcave *et al.*, *FEBS Letters*, **421**, 277-9 (1998)) and this should also have an anti-proliferative effect *in vivo*.

Resveratrol is a stilbene. It has two forms, the trans form and the cis form. A limited number of stilbene-containing plants have been consumed by man, and of these, the best known is the grape.

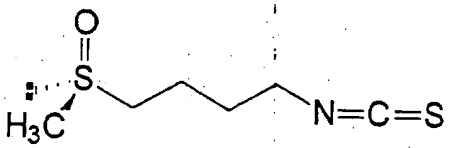




Several organic isothiocyanates have been tested for anti-cancer activity. One of these is sulforaphane which can be extracted from plants of the genus Brassica. It has been shown that sulforaphane does not induce phase I drug metabolising enzymes (Zhang et al(1992)). Later studies have shown that sulforaphane increased the activity of 2 isoforms of glutathione-S-transferase and decreased the major human cytochrome P450 CYP3A4 (Mahoe *et al.*, *Cancer Res.* 57, 3649-3652 (1998)). Sulforaphane has been reported to reduce the activation of the Aflatoxin B_1 by human hepatocytes (Longuet *et al. Molecular Toxicology* 11, 95-191, 1998) and to reduce the incidence and multiplicity of mammary tumours following administration of DMBA (Verhoeven *et al.*, 1997).

Some *in vivo* studies have also been performed on sulforaphane. Two studies showed a reduced binding of the aflatoxin B_1 following the administration of diets of cabbage and brussel sprouts. Reductions of DMBA or MNU-induced mammary tumours in rats have been found in 3 studies when cabbage, cauliflower, broccoli or brussel sprouts were included in the diet. Administration of cauliflower reduced the liver carcinogenesis induced by AFB1. A similar study, also with AFB1 showed a reduction in the number of tumours in the liver while a study on mice receiving cabbage along with DMH showed a reduction in the total number of tumours.

Sulforaphane is one of a number of organic thiocyanates released on hydrolysis of the aliphatic glucosinolates.



The present invention is based on the fact that a combination of resveratrol or its analogue and a brassica extract is surprisingly effective in treating tumours, especially testicular tumours. Accordingly, the present invention provides a composition suitable for pharmaceutical use which comprises at least one active ingredient obtainable from a brassica extract or an analogue of sulforaphane and resveratrol or an analogue thereof.

Resveratrol can also be obtained from plants. The composition can thus be obtained by mixing the plant extracts. The brassica extract may be obtained from any brassica vegetable which includes cabbage, kale, cauliflower, broccoli, mustard greens, kohlrabi, brussels sprouts and horseradish. The brassica extract is preferably a broccoli extract. Resveratrol may be extracted from grapes or other parts of grapevines or made via a synthetic preparation.

The analogues of resveratrol include stilbenes, hydroxylated stilbenes, for example trihydroxy-stilbenes and tetrahydroxy-stilbenes, which are typically phytoalexins, with or without one or more attached sugars or alkyl groups such as methyl; oligomers and/or polymers thereof, as well as oxidation or reduction products thereof. In particular, 3,4',5-trihydroxystilbene-3-beta-mono-D-glucoside or resveratrol which is preferred, may be used and the pharmacologically acceptable salts and esters thereof.

The sulforaphane analogue which can be used in the composition may be any compound having an isothiocyanate moiety and a polar functional group moiety,

wherein the two moieties are linked by a chain of one or more carbon atoms and the compound contains no pyridyl moieties, or a pharmacologically acceptable salt of such a compound.

The sulforaphane analogue is preferably not a heteroaromatic compound and is preferably not an arylalkyl compound. The analogue is preferably an olefin and is preferably aliphatic. The second moiety is preferably a polar functional group selected from a carboxylic ester, a carboxylic acid, a hydrocarbonoxy, a halogen, a hydroxyl, a ketone, a cyano, a nitro, a phosphine oxide, a sulfide, a sulfone, a sulfoxide, a thioether, and a thioester group, more preferably selected from a hydroxyl, a ketone, a phosphine oxide, a sulfone, and a sulfoxide group. The carbon chain of the sulforaphane component preferably comprises at least 3 carbon atoms, more preferably 3 to 5 carbon atoms. The carbon chain is preferably part of a non-aromatic ring.

The sulforaphane component is preferably selected from sulphoraphane itself, sulforaphene (4-isothiocyanato-(1R)-(methylsulfinyl)-1-(E)-butene), 6-isothiocyanato-2-hexanone, *exo*-2-acetyl-6-isothiocyanatonorbornane, *exo*-2-isothiocyanato-6-methylsulfonylnorbornane, 6-isothiocyanato-2-hexanol, 1-isothiocyanato-4-dimethylphosphonylbutane, *exo*-2-(1'-hydroxyethyl)-5-isothiocyanatonorbornane, *exo*-2-acetyl-5-isothiocyanatonorbornane, 1-isothiocyanato-5-methylsulfonylpentane and *cis*- or *trans*-3-(methylsulfonyl)cyclohexylmethylisothiocyanate and is preferably either form of sulforaphane, more preferably ((-) 1-isothiocyanato-(4R)-(methylsulfinyl)butane). Bertoin, alyssin, erucin, erysolin, iberverin, iberin and cheirolin may also be used.

Although the brassica extract and resveratrol and its analogues both show potential for reducing the incidence of cancers, surprisingly, when used together, they show a synergistic effect. The brassica extract or analogue of sulforaphane appears to act principally on the initiation phase of carcinogenesis. Whereas, resveratrol and its analogues may inhibit protein kinases *in vivo* and therefore affects the subsequent

proliferative phase of cancer.

A further aspect of the invention provides for the use of the composition of the invention for treating tumours. The composition can be used in a method of treatment of tumours.

In a further aspect, the composition may additionally comprise pharmaceutically acceptable diluents or excipients. It may also comprise antioxidant compounds, vitamins and minerals, in particular, vitamin A, vitamin C, vitamin E, lycopene and selenium.

The composition preferably comprises the active ingredient obtainable from a brassica extract or a sulforaphane analogue and resveratrol or its analogue in a weight ratio of 1:1000 to 1:10, preferably 1:500 to 1:50, more preferably 1:150 to 1:75 and especially about 1:100. This last formulation typically contains sulforaphane and resveratrol in a ratio of 2:1.

The composition is preferably administered in doses containing 1 to 100mg of the active ingredient obtainable from a brassica extract or sulforaphane analogue and 0.5 to 100mg of resveratrol or an analogue, preferably in a dose of 10mcg of active ingredient obtainable from a brassica extract or sulforaphane analogue : 1mg resveratrol or its analogues. The composition may be administered with a frequency of several times a day to once every two days, preferably daily. Treatment should be ongoing.

CLAIMS

1. A composition suitable for pharmaceutical use which comprises at least one active ingredient from a brassica extract or an analogue of sulforaphane, and resveratrol or an analogue thereof.

2. A composition according to claim 1 in which the resveratrol or analogue thereof is an extract of a grape or a grapevine

3. A composition according to claim 1 or 2 which comprises resveratrol.

4. A composition according to any one of the preceding claims which comprises sulforaphane.

5. A composition according to any one of the preceding claims which comprises brassica extract and in which the weight ratio of brassica extract to resveratrol or its analogue is from 1:500 to 1:50.

6. A composition according to any one of the preceding claims which comprises 1 to 100mg of the brassica extract or sulforaphane analogue and 0.5 to 100mg of resveratrol or analogue thereof.

7. A composition according to any one of the preceding claims which further comprises one or more of vitamin A, vitamin C, vitamin E, lycopene, lipoic acid, limonene, selenium and bromelain.

8. A composition according to claim 1 substantially as hereinbefore described.

9. A process for producing a composition according to any one of the preceding claims which comprises mixing the active ingredients together.

10. A composition according to any one of claims 1 to 8 for use in a method of treatment of the human or animal body by therapy.

11. Use of a composition according to any one of claims 1 to 8 in the manufacture of a medicament for use in the treatment of tumours.

12. Use according to claim 11 of a composition according to any one of claims 1 to 8 in the treatment of testicular tumours.

13. A product containing at least one active ingredient from a brassica extract or an analogue of sulforaphane, and resveratrol or an analogue thereof, for simultaneous, separate or sequential use in the treatment of tumours.

Application No: GB 9902304.6
Claims searched: 1-13

Examiner: Mrs Susan Chalmers
Date of search: 13 July 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): A5B: BJA

Int Cl (Ed.6): A61K: 31/05, 31/26, 31/70

Other: ONLINE: EPODOC, WPI, JAPIO, CHEMABS, BIOSIS, MEDLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	FEBS Letters vol 421 (1998) pages 277-279, M. Fontecave *et al*, "Resveratrol, a remarkable inhibitor of ribonucleotide reductase"	
A	Cancer Research vol 57 (1997) pages 3649-3652, K. Mahéo *et al*, "Inhibition of Cytochromes P-450 and Induction of Glutathione S-Transferases by Sulforaphane in Primary Human and Rat Hepatocytes"	
A	Science vol 275 (1997) pages 218-220, M Jang *et al*, "Cancer Chemopreventative Activity of Resveratrol, a Natural Product Derived from Grapes"	

X Document indicating lack of novelty or inventive step
Y Document indicating lack of inventive step if combined with one or more other documents of same category.
& Member of the same patent family

A Document indicating technological background and/or state of the art.
P Document published on or after the declared priority date but before the filing date of this invention.
E Patent document published on or after, but with priority date earlier than, the filing date of this application.